AN EXPERIMENTAL STUDY OF THE DETERIORATION AND ASSAY OF SPIRIT OF ETHYL NITRITE.*[1]

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INTRODUCTION.

Spirit of ethyl nitrite in the generally accepted sense is a solution of ethyl nitrite in strong alcohol. Although universally used in medical practice, the preparation is unsatisfactory as it undergoes rapid deterioration.

Since the first mention of a preparation of this nature in the literature by Raymond Lully in the 13th century, and subsequently by Basil Valentine in the 15th century (1), numerous efforts have been made to improve the keeping qualities of this product and thereby insure the uniformity of its potency. The efforts made along this line have in the main followed one of three courses, *viz.*, alteration of the method of preparation, addition of preservatives to the finished product, storage under special conditions.

Inasmuch as all three above-mentioned courses seemed to present opportunities worth studying, the experimental work undertaken and which is reported herein follows along these lines.

REVIEW OF WORK OF OTHER INVESTIGATORS.

Causes of Deterioration.—The deterioration of the spirit of ethyl nitrite consists in the main of the hydrolytic splitting of the ethyl nitrite with subsequent oxidation of the hydrolytic products and the re-union of some of these to form new compounds.

The normal constituents of the spirit are, ethyl nitrite, alcohol and water. On standing sensible amounts of nitrous, nitric and acetic acids are developed, also aldehyde, ethyl acetate and ethyl nitrate. Other compounds which have been identified in samples of the spirit examined and reported by various investigators are ethyl oxide, ethyl formate, ethyl oxalate, cyanides, glyoxal, glyoxalic acid, oxalic acid, malic acid and saccharic acid (2). Allen has also reported the presence of nitroethane, a substance isomeric with ethyl nitrate (3). The decrease in strength of the spirit is due to evaporation of ethyl nitrite as well as to the decomposition of the latter.

The exact conditions responsible for the destruction of the ethyl nitrite are not thoroughly understood, but it is established that the presence of an excess of water favors decomposition and that this decomposition is accelerated by exposure to air, sunlight and heat. The causes of decomposition as reported in the literature are as follows:

TABLE I.—REPORTED CAUSES OF DETERIORATION.

Year.	Investigator.	Cause of Deterioration.	Reference.
1823	Funcke	Addition of neutral potassium tartrate which causes the ethyl nitrite to separate from the spirit.	*Archiv. d. Pharm.*, 4, p. 344.
1824	Flashoff	Action of sunlight.	*Archiv. d. Pharm.*, 7, p. 61.

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[1] Section on Practical Pharmacy and Dispensing, A. PH. A., Miami meeting.

Year.	Investigator.	Cause of Deterioration.	Reference.
1827	Flashoff	Tendency to become acid.	*Archiv. d. Pharm.*, 21, p. 222.
1831	A. Duflos	Action of water.	*Archiv. d. Pharm.*, 36, p. 279.
1856	Kolbe	Water and oxygen of the air, producing nitrogen tetroxide, nitrous acid and nitric acid.	"Kolbe's Organ. Chem.," *Archiv. d. Pharm.*, 138, p. 164.
1885	G. E. Perry	High temperatures.	*Pharm. Jour.*, 45, p. 125.
1886	R. G. Eccles	Storage in large, partially filled containers.	PROC. A. PH. A., 34, p. 175.
1887	G. A. Atkinson	Water.	*Pharm. Jour.*, 47, p. 271.
1887	A. B. Lyons	Partially filled containers.	PROC. A. PH. A., 35, p. 624.
1893	Gibson	Glycerin in time, because of hygroscopic nature.	*Pharm. Jour.*, 52, p. 700.
1894	H. W. Jones	Water and evaporation.	*Pharm. Jour.*, 54, p. 163.
1894	Simpson	White bottles for storage.	*Pharm. Jour.*, 54, p. 163.
1894	J. S. Ward	Water.	*Pharm. Jour.*, 54, p. 164.
1900	Wilbur L. Scoville	Action of water in alcohol.	*Proc. Mass. State Pharm. Assoc.*, 1899, 53; PROC. A. PH. A., 48 (1900), 512.
1900	F. W. Pittuck and G. F. Merson	Evaporation and diffusion on continuous opening of bottle.	PROC. A. PH. A., 48 (1900), 511.
1902	S. F. Burford	Diffusion due to imperfect stoppering.	*Chem. & Drug.*, July 20, 1901, 108, through PROC. A. PH. A., 50 (1902), 749.
1902	E. H. Farr and R. Wright	Diffusion in partially filled bottles. Direct sunlight is fatal, but diffused light, however bright, has no chemical action on spirit.	*Trans. Brit. Pharm. Conf.*, 1901, 447, through PROC. A. PH. A., 50 (1902), 748.
1903	G. E. Shaw	Diffusion in partly filled, large bottles. Action of direct sunlight.	*Pharm. Jour.*, 8 (1903), 236; PROC. A. PH. A., 52 (1904), 564.
1911	Linwood A. Brown	Effect of actinic rays of light, volatilization, hydrolysis. Acid produced on standing accelerates deterioration.	*Amer. Drug.*, 10 (1911), 215; PROC. A. PH. A., 1911, 94.
1912	Southall's Report	Volatilization by mere pouring from one bottle to another. Hydrolysis practically nil in ten months.	*Southall's Report*, 1912, 20, 43, through "Brit. Y. B.," 1912, 335.
1913	R. C. Crowley	Evaporation in hot climates and hydrolysis when prescribed in aqueous solution.	"Proc. Austral. Pharm. Conf.;" *Austral. Jour. Pharm.*, 28 (1913), 19, through *Y. B., Brit. Phar. Assoc.*, 1913, 362.
1914	E. H. La Pierre	Heat, light and moisture.	"Proc. Mass. Phar. Assoc.," 1914, 88, through Y. B., A. PH. A., 3 (1914), 86.
1914	T. R. Hodgson	Evaporation in open and stoppered bottles.	*Pharm. Jour.*, 92, 28, through A. PH. A., 1914, 87.
1915	C. R. Marshall and Elizabeth Gilchrist	Evaporation in partially filled bottles, and opening bottle frequently.	*Brit. Med. Jour.*, 2 (1915), 125, through *Y. B., Brit. Phar. Assoc.*, 1916, 303.
1927–28	Senior Class of University of Philippines.	Air, light, water and kind of container.	*Jour. Philippine Phar. Assoc.*, Vol. 1, September 1928, 24.

The fact that the deterioration of the spirit is due in a large measure to the presence of water and is hastened by exposure to air, sunlight and high tempera-

ture, is fairly well established by the foregoing reports. It is confirmed by the results of the additional studies reported herein.

Methods Suggested for Preventing Deterioration.—The methods which have been suggested for preventing deterioration fall into two classes, *viz.:* those having as their main objective the prevention of loss of ethyl nitrite by evaporation, and those intended to prevent or retard destruction of the ethyl nitrite. The specific procedures recommended for accomplishing these purposes are summarized in the following table.

TABLE II.—SUGGESTED METHODS FOR PREVENTION OF DETERIORATION.

Year.	Investigator.	Preservative.	Reference.
1824	Flashoff	Spirit agitated for 18–20 days with MnO_2 and distilled slowly over MgO, does not become acid.	*Archiv. d. Pharm.*, 7, p. 61.
1824	Flashoff	Doubles amount of MnO_2 and prevents spirit from becoming acid.	*Archiv. d. Pharm.*, 9, p. 331.
1824	Th. Martius	Spirit distilled over KOH does not become acid within 6 months.	*Archiv. d. Pharm.*, 9, p. 331; *Buchn. Report.*, 15, p. 71.
1827	N. E. Henry	Magnesia, ferroso-ferric oxide, iron and copper prevent acidity when left in contact with spirit.	*Jour. de Pharmacie*, 13, p. 119.
1831	Braun	Shake with K_2CO_3 or MgO and keep over $CaCl_2$ to prevent acidity.	*Chem. Centribl.*, 2, p. 192.
1847	Klauer	Distilled over neutral potassium tartrate, yields a less acid product.	*Chem. Centribl.*, 19, p. 640.
1850	G. Reich	Acidity prevented by treating with neutral potassium tartrate, then preserving over calcium chloride or storing in 2- or 4-oz. bottles covered with gutta-percha.	*Archiv. d. Pharm.*, 112, p. 148.
1870	C. J. Rademaker	Potassium bicarbonate crystals, not so good tends to form nitrite.	*Am. Jour. Pharm.*, 42, p. 106.
1877	F. M. Rimmington	Strong alcohol essential to prevent decomposition.	*Pharm. Jour.*, 37, p. 453.
1885	W. H. Symons	Small bottles, well stoppered (paraffin covered) capped, wrapped in dark paper and stored in cool place.	*Pharm. Jour.*, 45, p. 164.
1886	J. Williams	Glycerin $^1/_3$, absolute alcohol $^2/_3$ as solvent.	*Pharm. Jour.*, 46, p. 255.
1887	G. A. Atkinson	Glycerin and acetate or citrate of ammonia retard decomposition.	*Pharm. Jour.*, 47, p. 271.
1888	T. S. Dymond and W. R. Dunstan	Absolute alcohol with 5% glycerin, best solvent.	*Pharm. Jour.*, 47, p. 790.
1892	W. Kinzel	Preserved over potassium tartrate.	*Pharm. Centrlhl.*, 33, p. 677.
1893	A. Meldrum	Addition of 10% glycerin.	*Chem. & Drug.*, 1893; *Amer. Jour. Pharm.*, 65, p. 199.
1893	Merck's Report	Sodium bicarbonate, potassium tartrate, magnesium carbonate, potassium bicarbonate have been used to neutralize the acid.	*Merck's Report*, 2, p. 22.
1893	Editor	Potassium tartrate best agent to preserve spirit.	*Pharm. Centrlhl.*, 34, p. 400.
1893	I. W. Thomson	5% glycerin in absolute alcohol is as stable as 10% glycerin in absolute alcohol.	*Pharm. Jour.*, 52, p. 699.

Year.	Investigator.	Preservative.	Reference.
1893	Gorrie	Preservative action of glycerin limited as to time (2 years).	*Pharm. Jour.*, 52, p. 700.
1893	Cowie	Glycerin in spirit acts as a preservative in lubricating the stopper, thus preventing access of air.	*Pharm. Jour.*, 52, p. 700.
1895	W. Smith	10% glycerin probably best preservative, but did not completely diminish loss in strength.	*Pharm. Jour.*, 54, p. 809.
1895	T. Dunlop	Should be kept in dark colored bottles excluded from light and heat.	*Chem. & Drug.*, 46, p. 70.
1898		5% glycerin.	*Pharm. Ztg.*, 43, p. 153.
1899	John Barclay	Sodium bicarbonate slightly retards loss of ethyl nitrite when bottle is well filled.	*Chem. News*, 12, 1899, 1030; PROC. A. PH. A., 48 (1900), 764.
1903	Frank Holman	Addition of simple syrup.	*Canad. Pharm. Jour.*, Aug. 1903, 20; PROC. A. PH. A., 52, 1903, 564.
1903	B. O. Leuber	Absolute alcohol with 5% glycerin. Spirit should be kept in well-filled, small, amber (not blue or green) bottles in a cool, dark place.	*Merck's Report*, Sept. 1902, 344.
1903	P. E. Hommell	Addition of small crystals of $KHCO_3$ to large quantities of spirit. Should be kept in small bottles.	*Proc. of N. J. Pharm. Assoc.*, 73, through PROC. A. PH. A., 51 (1903), 656.
1903	G. E. Shaw	Well-filled, small bottles kept in a cool place.	*Pharm. Jour.*, 8, 1903, 236. PROC. A. PH. A., 52 (1904), 564.
1911	J. Grier	Well-stoppered bottles essential.	*Chem. & Drug.*, 79 (1911), 537. *Y. B., Brit. Pharm. Assoc.*, 1912, 337.
1911	Linwood A. Brown	Spirit made with absolute alcohol stored in small, amber bottles below 10° C.	*Amer. Drug.*, 10, 1911, 215. PROC. A. PH. A., 1911, 94.
1912	Linwood A. Brown	Spirit made with absolute alcohol stored in small, amber bottles below 10° C., protected from light.	"Proc. Ky. Pharm. Assoc.," 1912, 134.
1912	Southall's Report	Glycerin has slight preservative action.	*Southall's Report*, 20 (1912), 43, through *Y. B., Brit. Phar. Assoc.*, 1912, 335.
1912	C. B. Jordan	Should be kept in 1- or 2-oz. bottles tightly stoppered and sealed with paraffin or wax.	"Proc. Ind. Pharm. Assoc.," 1912, 53, through Y. B., A. PH. A., 1 (1912), 58.
1913	R. C. Crowley	Glycerin and absolute alcohol in varying proportions.	Proc. Austral. Pharm. Conf.; *Austral. Jour. Pharm.*, 28 (1913), 19, through *Y. B., Brit. Phar. Assoc.*, 1913, 362.
1913	F. L. Shannon	Store not more than 6 months' supply in small, dark amber bottles in a cool place.	JOUR. A. PH. A., 1 (1913), 83.
1915	C. R. Marshall and Elizabeth Gilchrist	Ethyl nitrate diminishes loss of ethyl nitrite.	*Brit. Med. Jour.*, 2 (1915), 125, through *Y. B., Brit. Assoc. Phar.*, 1916, 303.
1917	T. C. N. Broeksmit	Bulk should be kept neutral by adding excess $MgCO_3$ and stored in a	*Pharm. Weekblad.*, 54 (1917), 1052.

		cool, dark place. For retail use filter and add Na_2SO_3	Y. B., A. PH. A., 6 (1917), 96. *Y. B., Brit. Phar. Assoc.*, 1918, 324.
1921	J. G. Roberts	Completely filled, small, amber bottles.	*Amer. J. Pharm.*, 93 (1921), 324. Y. B., A. PH. A., 10 (1921), 95.

The above suggestions have in the main been made with the best intentions, but the fact remains, that in actual practice the majority of them have been found to be of little or no value, and such of them as have been found to possess merit are only of value in so far as they diminish or retard deterioration. No method has yet been devised which will wholly prevent deterioration, nor has any practical means been found for preserving the product in a satisfactory condition for a reasonable period of time. The methods which have been found to give the best results will be discussed under the experimental work reported herein.

EXPERIMENTAL DATA.

As previously stated the purpose of the experimental work begun some two years ago and reported herein was to determine if it is possible to prepare a spirit of ethyl nitrite sufficiently stable to insure its potency over a reasonable length of time and the conditions which must be observed to give this result. The specific investigations undertaken were a study of the cause of deterioration from the standpoint of the effect of the various solvents used in the preparation of the spirit, the effect of added preservatives, the effect of colored glass containers and other conditions of storage. Incidentally, a comparison was made of the methods of assay as the official method, which is the one in general use in this country at the present time, it is inconvenient and not wholly satisfactory in other respects.

Preparation of Samples.—The spirit used in all of the experiments conducted in this investigation was prepared according to the formula given in the ninth edition of the United States Pharmacopœia (4). It was assayed for ethyl nitrite content immediately after preparation. In those cases where a large number of bottles were filled for use in a series of assays, the spirit was first assayed and then transferred to the various containers, the temperature being maintained below 5° C. during the transfer. Where only a few bottles were filled, the contents of each bottle were assayed separately.

In all cases, the solution was transferred immediately to glass bottles tightly stoppered with the best grade of cork. The stoppers were twisted in where the bottles were placed in the refrigerator or on the laboratory shelf, but were all securely tied in by means of heavy twine when they were placed in direct sunlight. When the stoppers became spongy or in any way affected by the spirit they were replaced by new ones. The term refrigerator in Table IX refers to one cooled by ice, while the same term in all other tables refers to an electric refrigerator. Direct sunlight implies that the bottles were placed on the roof of the laboratory building in such position that they were exposed to the rays of the sun from sunrise to sunset, and exposed to all kinds of weather. To determine the effect of diffused sunlight, the bottles were kept on a laboratory shelf out of the direct rays of the sun.

COMPARISON OF THE EFFECT OF DIFFERENT SOLVENTS ON THE RATE OF DETERIORATION.

Inasmuch as the work of other investigators has shown that the deterioration of the spirit is influenced in some degree by the solvent used in making the preparation, the following experiments were undertaken. The possibilities in this field are necessarily limited on the one hand to those substances in which ethyl nitrite is soluble, and on the other hand to those which have little or no effect on the therapeutic use.

Throughout this series of experiments the strength of ethyl alcohol where used is expressed in percentage by volume. In addition to using 95 per cent ethyl alcohol, which is the solvent specified in the U. S. Pharmacopœia, 99 per cent, 90 per cent and 75 per cent ethyl alcohol were used, the ethyl nitrite content being kept within the pharmacopœial limits, namely, 3.5 per cent and 4.5 per cent (5). Alcohols of these strengths were used to determine the effect of the presence of different amounts of water on the rate of decomposition. The official diluted ethyl alcohol (6) (49 per cent by volume) was also tried, but was discarded as ethyl nitrite was found not to be soluble to the extent of 3.5 per cent therein.

The iso-propyl alcohol used was of the quality labeled chemically pure and obtained from The Coleman & Bell Company, Norwood, Ohio. It was tried because it is free of the legal restrictions surrounding ethyl alcohol, is fairly cheap, possesses solvent properties similar to ethyl alcohol and contains but little water.

Some samples were also prepared with a non-volatile solvent, liquid petrolatum, as it was thought that the rate of evaporation of the ethyl nitrite might be retarded by this means and that hydrolytic decomposition might also be less likely to occur. The heavy liquid petrolatum used was that supplied retail pharmacy. Other non-volatile solvents which suggested themselves, namely, glycerol and ethylene glycol were also tried but it was found that ethyl nitrite was not sufficiently soluble in either of them to justify their use. The following tables give the results of these attempts.

TABLE III.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	4.23	4.23	4.23	4.23	4.23
7/8/30	4.12	0.42*	4.03	3.99	3.98
7/11/30	4.04	0.07	3.99	3.97	3.96
7/16/30	3.99	0.00	3.98	3.90	3.92
8/8/30	3.91	...	3.87	3.80	3.86
9/18/30	3.78	...	3.52	3.76	3.59[4]
12/5/30	3.44*	...	2.66*	3.37*	3.22*,[5]
2/13/31	3.19	...	2.23	3.29	3.07
3/13/31	3.14	...	2.09	3.09	2.98
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	5.68	100.00	5.90	7.81	7.33
3/13/31	25.80	...	50.60	27.00	29.59

* Below U. S. P. minimum requirement.

[1] Kept in refrigerator at 7° C. to 9° C.

TABLE IV.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (99%).

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	4.45	4.45	4.45	4.45	4.45
7/8/30	4.29	1.34*	4.36	4.25	4.36
7/11/30	4.27	0.11	4.27	4.21	4.31
7/16/30	4.26	0.00	4.18	4.15	4.29
8/8/30	4.20	...	3.88	4.08	4.18
9/18/30	4.13	...	3.41*	3.93	4.17
12/5/30	4.09	...	2.86	3.56	4.09
2/13/31	4.00	...	2.73	2.05*,[4]	3.92
3/13/31	3.83	...	2.67	1.95	3.89
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	4.27	100.00	6.07	6.74	3.59
3/13/31	11.68	...	40.00	56.2	12.57

TABLE V.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (90%).

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	4.33	4.33	4.33	4.33	4.33
7/8/30	4.18	0.12*	4.22	4.25	4.21
7/11/30	4.09	0.02	4.17	4.25	4.14
7/16/30	4.05	0.00	4.16	4.01	4.14
8/8/30	4.04	...	4.09	3.86	3.98
9/18/30	3.76	...	3.43*	3.45*	3.60
12/5/30	3.72	...	2.64	3.31	3.21*
2/13/31	3.25*	...	2.60	2.49	2.80
3/13/31	3.14	...	2.09	3.09	2.98
		Loss of Ethyl Nitrite in Per Cent.			
7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	6.47	100.0	3.93	7.41	4.17
3/13/31	27.75	...	43.70	53.80	40.00

TABLE VI.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (75%).

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	3.75	3.75	3.75	3.75	3.75
7/8/30	3.69	0.69*	3.58	3.54	3.61
7/11/30	3.55	0.08	3.46*	3.40*	3.46*
7/16/30	3.52	0.00	3.32	3.34	3.27
8/8/30	3.26*	...	2.74	3.06	3.01
9/18/30	3.21	...	2.12	2.89	2.75
12/5/30	3.15	...	1.81	2.26	1.73
2/13/31	2.40	...	1.45	1.29	1.20
3/13/31	2.12	...	1.38	1.29	1.11

[2] Kept out of doors in direct sunlight, both summer and winter, the temperature ranging from 40° C. in summer to −10° C. in winter.

[3] Kept in the laboratory at room temperature at 22° C. to 28° C.

[4] Stopper in spongy condition when removed from bottle.

[5] Stopper broke and part fell in spirit requiring a transfer into another bottle.

Loss of Ethyl Nitrite in Per Cent.

7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	6.14	100.00	8.81	10.94	12.79
3/13/31	43.45	...	72.00	60.45	70.5

TABLE VII.—SOLUTION OF ETHYL NITRITE IN ISO-PROPYL ALCOHOL (C.P.).

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	4.33	4.33	4.33	4.33	4.33
7/8/30	4.31	0.36*	4.20	4.12	4.28
7/11/30	4.19	0.03	4.09	3.99	4.16
7/16/30	4.14	0.00	4.00	3.96	3.87
8/8/30	4.02	...	3.77	3.37*	3.49*
9/18/30	3.93	...	3.17*	3.05	3.09
12/5/30	3.27*	...	2.95	2.71	2.83
2/13/31	3.00	...	2.62	1.11	2.65
3/13/31	2.90	...	2.55	0.91	2.55

Loss of Ethyl Nitrite in Per Cent.

7/3/30	0.00	0.00	0.00	0.00	0.00
7/16/30	4.38	100.00	7.62	8.54	10.61
3/13/31	33.05	...	41.23	79.10	41.23

TABLE VIII.—SOLUTION OF ETHYL NITRITE IN SQUIBB'S LIQUID PETROLATUM.

Assay Results.

Date.	Refrigerator.[1] White glass, per cent.	Direct Sunlight.[2] White glass, per cent.	Direct Sunlight.[2] Amber glass, per cent.	Diffused Sunlight.[3] White glass, per cent.	Diffused Sunlight.[3] Amber glass, per cent.
7/3/30	4.46	4.46	4.46	4.46	4.46
7/8/30	4.23	2.28*	4.19	4.20	4.39
7/11/30	4.18	0.99	4.11	4.14	4.18
7/18/30	4.16	0.00	3.83	3.75	3.87
8/8/30	4.11	...	3.76	3.65	3.74
9/18/30	4.05	...	3.45*	3.59	3.61
12/5/30	3.89	...	3.25	3.29*	3.56
2/12/31	3.33*	...	2.91	3.21	3.35*
3/13/31	3.27	...	2.73	2.69	3.16

Loss of Ethyl Nitrite in Per Cent.

7/3/30	0.00	0.00	0.00	0.00	0.00
7/18/30	6.71	100.00	14.09	15.88	13.21
3/13/31	26.61	...	38.79	39.64	29.04

The results of these experiments show that 99 per cent ethyl alcohol is the best solvent for use under general conditions. It is followed closely by 95 per cent ethyl alcohol, but beyond this dilution the rate of decomposition is so rapid that the preparation is wholly unsatisfactory.

Iso-propyl alcohol proved to be unsatisfactory as Table VII shows, the rate of deterioration of the spirit made with this solvent being about equal to that made with ethyl alcohol of 90 per cent.

Heavy liquid petrolatum as shown in Table VIII indicates that this substance might be a suitable solvent under certain conditions, as the rate of deterioration of the ethyl nitrite in solution is about equal to that of the preparation made with 95 per cent ethyl alcohol. Its principle disadvantage is that it is immiscible with practically all of the preparations that are ordinarily prescribed in

combination with the spirit. It is also unsatisfactory from the standpoint of storage as diffusion of the products of decomposition, as indicated by destruction of cork stoppers, is greater than where ethyl alcohol is the solvent used. The solution prepared with this solvent becomes dark brown and finally black on storage.

COMPARISON OF THE INFLUENCE OF DIFFERENT COLORED CONTAINERS ON THE RATE OF DETERIORATION.

Previous work reported in the literature has shown that exposure of the spirit to sunlight accelerates deterioration, and that this deterioration seems to be directly proportional to the length of time exposed (7). It has further been shown that the actinic rays (8) are the ones most active in effecting decomposition. This being true, it is only logical to conclude that deterioration may be retarded by shielding the preparation from these rays. With this purpose in view, samples of the spirit were stored in bottles made of different colored glass, the colors selected being, blue and amber. The spirit of ethyl nitrite thus bottled was stored side by side with a sample contained in a clear glass bottle for comparison. The results obtained were as follows:

TABLE IX.—SOLUTION OF ETHYL NITRITE IN ETHYL ALCOHOL (95%).

Assay Results.

	Refrigerator.[6]			Direct Sunlight.[7]			Diffused Sunlight.[7]		
Date.	White, per cent.	Blue, per cent.	Amber, per cent.	White, per cent.	Blue, per cent.	Amber, per cent.	White, per cent.	Blue, per cent.	Amber, per cent.
8/16/29	4.32	4.32	4.32	4.32	4.32	4.32	4.32	4.32	4.32
8/17/29	...	...	...	3.88	3.94	4.32	...	...	...
8/19/29	4.10	4.18	4.12	3.82	3.74	4.32	4.11	4.19	4.21
8/20/29	...	...	...	3.49*	3.38*	3.94	...	...	...
8/21/29	...	...	...	3.02	3.15	3.86	...	...	...
8/22/29	...	...	...	2.96	2.54	3.84	...	...	...
8/23/29	4.04	4.13	4.09	2.50	2.31	3.82	4.06	4.09	4.04
8/24/29	...	...	...	2.36	2.23	3.76	...	...	...
8/26/29	4.02	4.09	4.06	1.59	1.70	3.71	3.93	3.99	4.03
8/28/29	3.89	3.69	3.97	0.31	0.38	3.65	3.90	3.78	3.94
3/11/30	...	...	...	0.00	0.00	1.71*	3.38*	3.44*	3.46*
				Loss of Ethyl Nitrite in Per Cent.					
8/16/29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8/17/29	...	...	...	10.18	8.8	0.00	...	...	...
8/19/29	5.09	3.24	4.63	11.52	13.41	0.00	4.86	3.04	2.55
8/20/29	...	...	...	19.18	21.65	8.78	...	...	...
8/21/29	...	...	...	30.05	27.06	10.62	...	...	...
8/22/29	...	...	...	31.42	41.16	11.09	...	...	...
8/23/29	6.47	4.39	5.31	42.05	46.50	11.48	6.00	5.32	6.47
8/24/29	...	...	...	45.38	48.40	12.92	...	...	...
8/26/29	6.95	5.32	6.00	63.00	60.50	14.10	9.03	7.62	6.71
8/28/29	9.95	14.50	8.09	92.85	90.95	17.79	9.72	12.48	8.78
3/11/30	...	...	...	100.00	100.00	60.45	21.68	20.28	19.85

[6] Kept in refrigerator at 9° C. to 12° C.

[7] Kept out of doors in direct sunlight, the temperature ranging from 15° C. to 35° C.

The degree of protection afforded by colored glass containers, as revealed in the above experiment, is in the order amber, blue and clear glass. In fact the protection afforded by blue glass is practically nil, as the rate of deterioration of the spirit in blue glass containers is almost as rapid as that in clear glass containers.

(*To be continued*)